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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY
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THE 'REFRACTIVE BODY' AND THE 'MITOCHONDRIA' OF ASCARIS CANIS WERNER.

BY A. C. WALTON.

WITH TWO PLATES

(Continued from page 3 of cover.)

VOLUME 52.

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THE 'REFRACTIVE BODY' AND THE 'MITOCHONDRIA' OF ASCARIS CANIS WERNER.

By A. C. Walton.

Received June 2, 1916. Presented by E. L. Mark.

The work of Mayer ('08), Romieu ('11), Meves ('11), Fauré-Frémiet ('11), Wildman ('12) and Romeis ('12) on Ascaris megalocephala, and that of Marcus ('06) on Ascaris canis, has shown that the refractive body of the sperm is formed in the vas deferens shortly before the process of copulation takes place. Their work has also shown that the cytoplasmic granules which surround the nuclei of the nuclei of the spermatids are the so-called 'mitochondria.' Marcus, working on what he supposed to be A. canis, was the first to recognize the source of the refractive bodies. The present writer has elsewhere shown that the form worked on by Marcus was, however, not A. canis but another, as yet unplaced, nematode. It remained for Wildman to follow out more completely the history of the formation of the 'refractive body' and its relation to the 'mitochondria' in A. megalocephala and to show that Marcus's conjecture was an actual fact. Inasmuch as the work of Marcus was not, after all, on A. canis, a description of the conditions in that species may have some value.

The writer here wishes to express his thanks to Dr. S. I. Kornhauser of Northwestern University for assistance in his earlier work on A. canis, and also to Dr. E. L. Mark for his helpful advice and criticism in the completion of this paper.

'The Refractive Body.'

Marcus in A. canis, and Mayer, Romieu and Wildman in A. megalocephala, have shown that the refractive body is formed by the fusion of the 'refringent vesicles' of the spermatocytic stages. Wildman alone gives an account of the formation of the refringent vesicles, or

granules; he thus completes the history of the formation of the refractive body. The writer has attempted to follow out the history of this body in the case of A. canis and to ascertain if in that species Wildman's conclusions are realized.

Technique.

The material used for this work was fixed in Flemming's (strong) or in Carnoy's fluid. Some of the material was stained with iron haematoxylin-Bordeaux red, some with Ehrlich-Biondi stain, and some according to Benda's method. By these stains it was easy to distinguish between the materials of cytoplasmic and karyoplasmic origin, since they took decidedly different colors.

Origin of the 'refringent vesicles.'

The cytoplasm of the early spermatogonia does not show any of the bodies which take the blue color in Benda's stain ('karyochondria' of Wildman), but the nucleus does show numerous such particles. No evidence was found, such as that given by Wildman, to show that this material covered the surface of the karyochromatin. These separate particles were clearly distinguishable from the karyosome masses and the plastosome (Fig. 1). In the older spermatogonia and in the youngest spermatocytes, bodies staining blue like those of the nucleus appeared in the cytoplasm and at the same time the number of such particles in the nucleus was greatly reduced (Fig. 2). No direct evidence of the actual migration of the particles from the nucleus into the cytoplasm, such as shown by Wildman, Figure 4, to be the case in A. megalocephala, was found in A. canis. Little doubt as to the identity of the particles in the nucleus and in the cytoplasm can be entertained, since the staining reactions of the two groups of granules are identical and the increase of one comes at the time of a great decrease in the numbers of the other. These small cytoplasmic bodies are the primitive 'refringent granules,' which are therefore nuclear in origin. These bodies correspond to the 'Trophochromatin' of Marcus and the 'karyochondria' of Wildman. They swell up by the accumulation of fat, thus forming the 'refringent vesicles' that are so prominent, even in the living material, during the late spermatocytic and early spermatid stages.

Fate of the 'refringent vesicles.'

During the first maturation division, the 'vesicles' arrange themselves in radiating rows parallel to the astral rays and each assumes an oval form (Figs. 3 and 4). In the center of each of these granules, after careful destaining, can be seen short, deeply staining, granular rod-like bodies (Fig. 5) that run lengthwise of the vesicle. The beginning of the second maturation division finds the elongation of the refringent vesicles very pronounced, the vesicles being from three to four times as long as broad. As the second division goes on, the vesicles take a very marked peripheral position with their long axes perpendicular to the cell membrane (Figs. 6 and 7), leaving a clear perinuclear space filled with small granules. These are the 'microsomes' of Van Beneden and the 'plastochondria' of later writers. At the same time the rod-like granular bodies within the refringent vesicles disappear. Both Meves and Romeis have clearly shown that these bodies and the 'plastochondria' are identical structures in the case of A. megalocephala. They certainly have the same staining reactions in Benda's and Ehrlich-Biondi stains, and there can be little doubt but that they are also identical with the bodies seen in A. megalocephala.

The process of 'cytoplasmic reduction' takes place shortly after the second division has been completed, but no refringent vesicles are lost with the extruded cytoplasm, only a considerable number of the small granules derived from the vesicles at the time of the second division. The spermatid is thus reduced by at least one third of its original volume, the refringent vesicles becoming very closely packed together. The vesicles now fuse into irregularly shaped bodies that soon round up into highly refractive spheres (Figs. 8 and 9). These larger spheres again fuse with one another, forming a hollow sphere of globules surrounding the nucleus (Fig. 10). The globules, in undergoing further fusion, move towards one end of the spermatid, leaving the nucleus and its surrounding plastochondria lying free in the cytoplasm (Fig. 11). In Benda's stain these globules now begin to lose the blue color and to take on the yellow stain of yolk material, showing that the karyochondrial material is gradually being changed into food. Fusion continues until a single hemispherical body, slightly concave on the side next to the nucleus, is formed (Fig. 12). This body is the 'refractive body' and is fully formed in the mature spermatozoön just before copulation takes place. Its chemical make-up is almost entirely that of yolk material.

After copulation, the amoeboid spermatozoön makes its way up to the proximal end of the uterus before insemination can take place. Here, as Wildman and others have shown in A. megalocephala, it is found that in about 90 per cent of the spermatozoa the refractive bodies have degenerated, and often are entirely wanting (Figs. 13 and 14). As many spermatozoa completely lack the refractive body as have it in a fully developed condition. That this appearance is not due to a degeneration of the sperm is demonstrated by the fact that more eggs were found penetrated by sperms in this condition than by sperms having a fully developed refractive body. It seems to the writer that, as Marcus has hinted and Wildman definitely stated, the function of the refractive body is not one of a mechanical support for the sperm head in penetrating the egg, but rather that of a source of food during the long interval between copulation and insemination. If this view is correct, the food of the spermatozoön is provided by the extrusion of a nuclear substance in the early spermatocyte and a building up of a food supply through the action of this material upon the cytoplasm of the spermatocyte and of the spermatid.

The 'Mitochondria.'

With iron haematoxylin-Bordeaux red stain the plastosome and other scattered nuclear granules take a red stain, while the karyosome and its derivatives take a blue stain. These granules later (early spermatocytes) disappear from the nucleus simultaneously with the disappearance of the plastosome. At the same time similar granules are found in the 'refringent vesicles' and continue to show quite plainly up to the time of the second division (Fig. 5). As Montgomery ('11) has shown in Euschistus, the plastosome is of karyosomal origin; and as the refringent vesicles of Ascaris are karyochondria, the statement of Wildman, that the granules of the refringent vesicles are also of karyosomal origin seems most probable.

As mentioned above, at the anaphase of the second maturation division, these granules are given off by the refringent vesicles as the plastochondria. The majority of these granules gather in the perinuclear space, but a large number of the smaller ones are also found scattered generally throughout the cytoplasm of the cell (Fig. 6) and hence are lost at the time of the 'cytoplasmic reduction.' Wildman states that these granules fuse to a slight extent, but no direct proof of this could be found in A. canis, although there are fewer and larger

granules at the time of the completion of the refractive body than there were in the early spermatids. The greater number of these granules, or plastochondria, are clustered around the nucleus, although a few of the smaller ones are found scattered throughout the cytoplasmic sheath that covers the refractive body (Fig. 12). These are most plainly seen in spermatozoa in which the refractive body has been partially or entirely used up (Figs. 13 and 14). After having been carried into the egg with the sperm, the larger of the plastochondria can be distinguished for some time after the sperm nucleus has left them and set up its own vesicle preparatory to uniting with the female pronucleus (Fig. 15). These granules slowly fuse with, and become indistinguishable from, the granules of the egg cytoplasm by the time the two pronuclei have united, although occasional cells show a few of the plastochondria still distinguishable at this time (Fig. 16).

Meves believes that these plastochondria are the 'plasma bearers of heredity' because they fuse with similar granules in the egg cytoplasm. In order to prove that they may be such 'bearers,' satisfactory answers must be found to several perplexing questions that are as yet unanswered. One of these is the question as to the nature of the 'cytoplasmic reduction.' In this reduction a great many of the plastochondria are lost. To prevent the loss of parental qualities borne by these granules it must be that there either is a selective division,— so that only plastochondria bearing duplicate characters are cast off,— or else the larger granules, which remain near the nucleus and are never lost, are the only ones that carry hereditary qualities. The first supposition is not substantiated by facts thus far brought out and hence must be abandoned. The second supposition, that the hereditary qualities are borne by the larger plastochondria, has been invalidated by the work of Vejdovský, in which he has shown that, inasmuch as these plastochondria entirely lose their identity, they are in no way continuous from generation to generation and hence can not be bearers of hereditary qualities.

The work of Lillie on Nereis is also an argument against the functioning of the plastochondria as bearers of hereditary qualities, for he finds that the plastochondria of the sperm do not even enter the egg, but, with the tail-piece, remain outside. The classical work of Boveri on the fertilization of enucleated egg fragments also shows that the plastochondria of the female do not carry hereditary qualities. These granules unite with the corresponding ones of the male. Now, if the latter carry male characteristics, we should be justified in supposing that the female granules would similarly carry female characters, since

the offspring of normal unions have qualities of both parents. If, however, the female plastochondria do not carry such determinants, as Boveri's work shows, then we are not justified in supposing that the male plastochondria do.

From this evidence it seems clear that the plastochondria, or the so-called 'mitochondria,' can not be true 'plasma bearers of heredity,' at least in the nematodes Ascaris canis and Ascaris megalocephala, as well as in the cases studied by Lillie and by Boveri.

Summary.

(1) The 'refractive body' is formed by the fusion of the 'refringent vesicles.'

(2) The 'refringent vesicles' are formed from the cytoplasm of the spermatocytes through the action of small extruded granules of karyochromatin, the 'karyochondria' of Wildman.

(3) The 'refractive body' in Ascaris canis Werner takes no part in the fertilization of the egg other than as a source of food supply to the spermatozoön between the time of copulation and the time of insemination.

(4) The 'plastochondria' are partly of plastosomal and partly of karyochondrial origin through their formation in the 'refringent granules.'

(5) The 'plastochondria' (mitochondria) are not 'plasma bearers of heredity' in Ascaris canis Werner.

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DESCRIPTION OF PLATES.

All figures are camera drawings, made with a Spencer microscope; achromatic, homog. immersion lens, 1.8 mm., N. A. 1.30; tube length 165 mm.; and projection distance 420 mm.

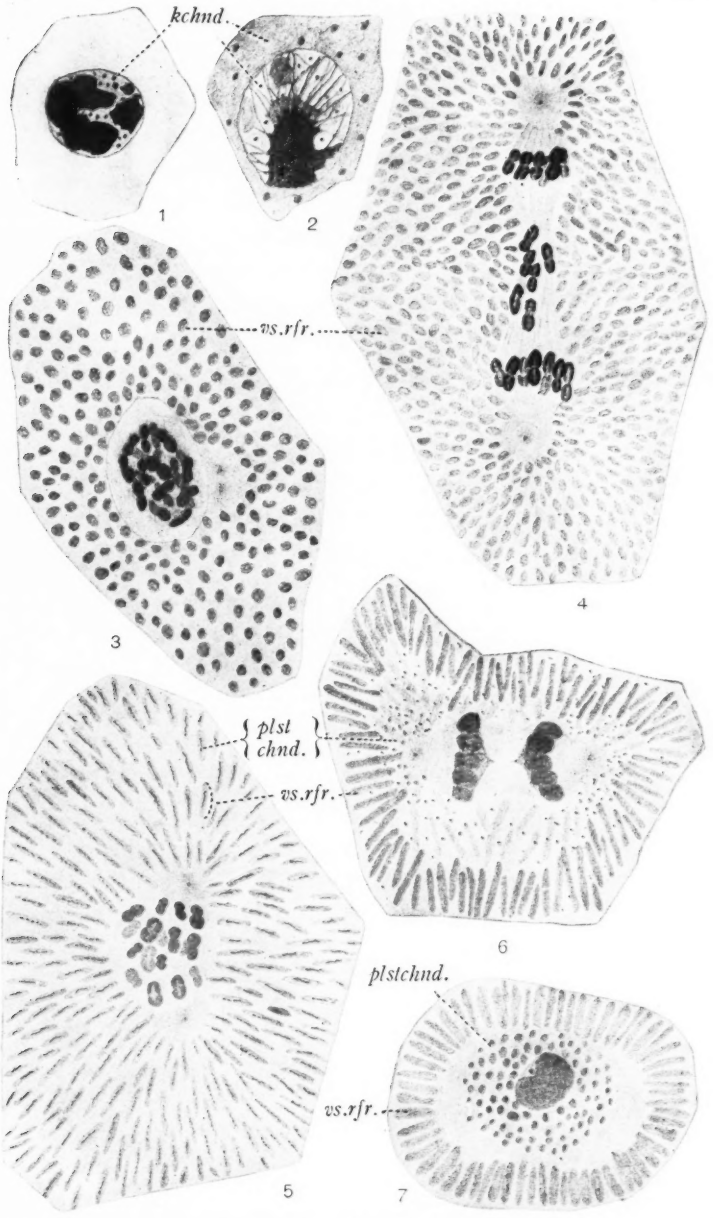
Figures 1–14 are reproduced at an enlargement of 4,000 diameters (compensating ocular 18x, Zeiss); Figures 15 and 16 an enlargement of 1700 diameters (compensating ocular 9x, Zeiss). In reproducing Figures 15 and 16 they were reduced to $\frac{2}{3}$ original size, so that their magnification on the plate is 1133 diameters.

Abbreviations.

cp. rfr.	refractive body
kchnd.	karyochondria
plstchnd.	plastochondria
vs. rfr.	refringent vesicles

PLATE 1.

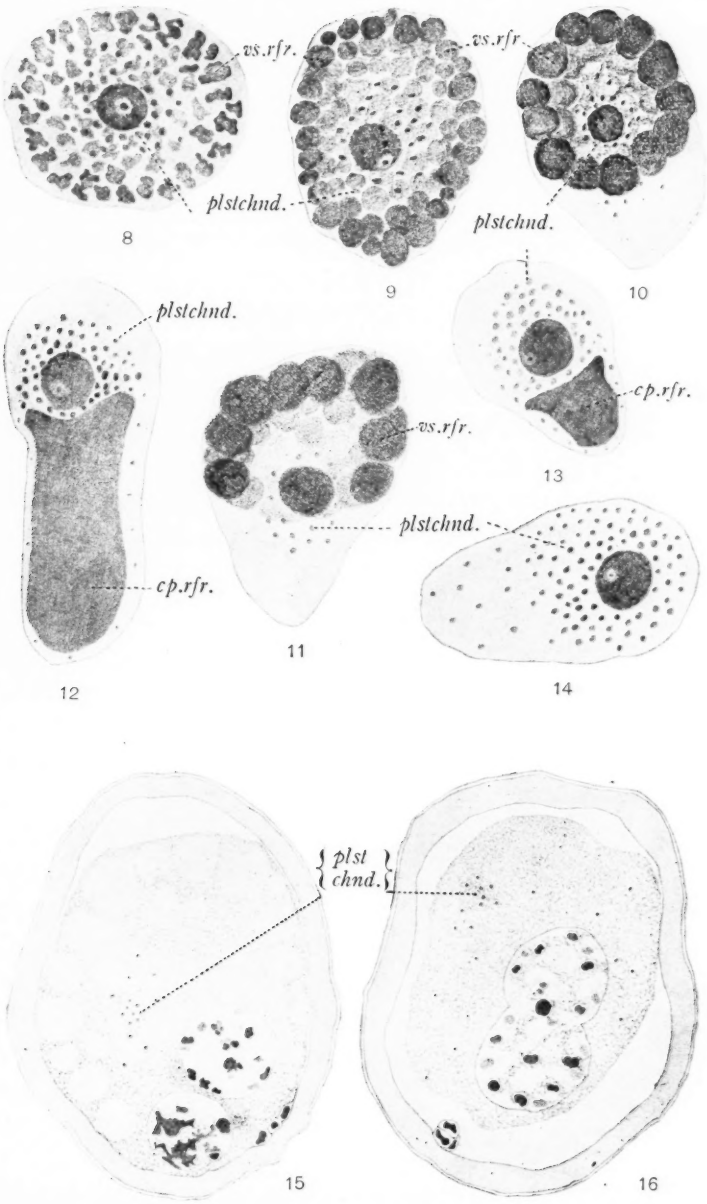
FIGURE 1. Spermatogonium showing intra-nuclear karyochondria (*kchnd.*).
FIGURE 2. Early spermatocyte showing extra-nuclear distribution of karyochondria.
FIGURE 3. Late prophase, first division, showing refringent granules (*vs.rfr.*) developed from karyochondria.
FIGURE 4. Anaphase, first division, showing radial arrangement of 'refringent vesicles' (*vs. rfr.*).
FIGURE 5. Prophase, second division, showing extreme elongation of 'refringent vesicles' and their contained plastochondrial, granular rods (*plstchnd.*).
FIGURE 6. Anaphase, second division, showing peripheral position of 'refringent vesicles' and also the plastochondria, now lying free in the cytoplasm.
FIGURE 7. Early spermatid, showing peripheral position of 'refringent vesicles' and the large plastochondria in the perinuclear space.



A. C. W. DEL.

PLATE 2.

FIGURE 8. Spermatid, showing fusion of the 'refringent vesicles.'
FIGURE 9. Spermatid, showing advanced fusion of the 'refringent vesicles.'
FIGURES 10 and 11. Early spermatozoa, showing the withdrawal of the 'refringent vesicles' to one end of the cell and their further fusion, leaving the nucleus and surrounding plastochondria free in the cytoplasm.
FIGURE 12. Mature spermatozoön at time of copulation, showing refractive body (*cp. rfr.*) formed by the fusion of the 'refringent vesicles.'
FIGURE 13. Mature spermatozoön after remaining for some time in oviduct of female, showing shrinkage in size of the nutritive refractive body (*cp. rfr.*).
FIGURE 14. Mature spermatozoön, at time of insemination, which has entirely used up refractive body. Plastochondria are scattered all through the cytoplasm.
FIGURE 15. Fertilized egg with pronuclei not as yet united, showing persistence of male plastochondria.
FIGURE 16. Fertilized egg with pronuclei uniting, showing last remnants of male plastochondria, the most of which are scattered throughout the cell.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